



APPENDIX A

LISTING OF CLAIMS

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Claim 3. A method for binding a DNA binding polypeptide of the Cys2

His2 zinc finger class to a DNA triplet in a target DNA sequence comprising 5-meC as the central residue in the target DNA triplet, the method comprising preparing a DNA binding polypeptide of the Cys2 His2 zinc finger class to bind to the DNA triplet, wherein binding to the 5-meC residue by an α -helical zinc finger DNA binding motif of the polypeptide is achieved by placing an Ala residue at position +3 of the α -helix of the zinc finger, and exposing the DNA binding polypeptide to the target DNA sequence, whereby the DNA binding polypeptide binds to the target DNA sequence.

Claim 4. A method for binding a DNA binding polypeptide of the Cys2

His2 zinc finger class to a DNA triplet in target DNA sequence comprising 5-meC, but not to an identical triplet comprising unmethylated C, the method comprising preparing a DNA binding polypeptide of the Cys2 His2 zinc finger class to bind to the triplet comprising 5-meC, wherein binding to each base of the triplet by an α -helical zinc finger DNA binding motif in the polypeptide is determined as follows:

- a) if the 5' base in the triplet is G, then position +6 in the α -helix is Arg or position ++2 is Asp, or position +6 in the α -helix is Arg and position ++2 is Asp;
- b) if the 5' base in the triplet is A, then position +6 in the α -helix is Gln or Glu and ++2 is not Asp;
- c) if the 5' base in the triplet is T, then position +6 in the α -helix is Ser or Thr and position ++2 is Asp; or position +6 is a hydrophobic amino acid other than Ala;
- d) if the 5' base in the triplet is C, then position +6 in the α -helix is any amino acid, provided that position ++2 in the α -helix is not Asp;
- e) position +3 in the α -helix is Ala;
- f) if the 3' base in the triplet is G, then position -1 in the α -helix is Arg;
- g) if the 3' base in the triplet is A, then position -1 in the α -helix is Gln and position +2 is Ala;
- h) if the 3' base in the triplet is T, then position -1 in the α -helix is Asn; or position -1 is Gln and position +2 is Ser;

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i) if the 3' base in the triplet is C, then position -1 in the α -helix is Asp and Position +1 is Arg; and

exposing the DNA binding polypeptide to the target DNA sequence, whereby the DNA binding polypeptide binds to the target DNA sequence.

Claim 23. The method according to claim 3 or 4, wherein the binding protein comprises two or more zinc finger binding motifs.

Claim 25. The method according to claim 23, wherein the DNA binding protein is constructed by recombinant DNA technology, the method comprising the steps of:

- a) preparing a DNA coding sequence encoding two or more zinc finger binding motifs;
- b) inserting the DNA sequence into a suitable expression vector; and
- c) expressing the DNA sequence in a host organism in order to obtain the

DNA binding protein.

Claim 26. The method according to claim 3 or 4 further comprising the steps of subjecting the DNA binding protein to one or more rounds of randomization and screening in order to improve the binding characteristics thereof.

Claim 28. The method of either of claims 3 or 4, further comprising detecting the DNA binding polypeptide binding to the target DNA sequence.

Claim 29. The method of either of claims 3 or 4, wherein the binding of the DNA binding polypeptide to the target DNA sequence regulates transcription of a gene.